

# Differential effects of petit mal anticonvulsants and convulsants on thalamic neurones: calcium current reduction

<sup>1</sup>Douglas A. Coulter, John R. Huguenard & David A. Prince

Department of Neurology and Neurological Sciences, Rm M016, Stanford University Medical Center, Stanford, CA 94305, U.S.A.

1 Succinimide derivatives can be either convulsant (tetramethylsuccinimide (TMS)), or anticonvulsant (ethosuximide (ES);  $\alpha$ -methyl- $\alpha$ -phenylsuccinimide (MPS)). ES, an anticonvulsant succinimide, has previously been shown to block calcium currents of thalamic neurones, while the convulsant succinimide TMS blocks  $\gamma$ -aminobutyric acid (GABA) responses in a similar fashion to the convulsant pentylenetetrazol (PTZ).

2 Using voltage-clamp techniques, we analysed the effects of the anticonvulsant succinimides ES and MPS and the convulsants TMS and PTZ on calcium currents of acutely isolated thalamic relay neurones of the rat.

3 MPS and ES reduced low-threshold calcium current (LTCC) in a voltage-dependent manner, without affecting steady-state inactivation. MPS was less potent than ES ( $IC_{50}$  of 1100 vs 200  $\mu$ M) but greater in efficacy (100% maximal reduction vs 40% for ES).

4 PTZ had no effect on calcium currents, and TMS only reduced LTCC at very high concentrations, and did not occlude MPS effects when applied concurrently.

5 These results, which demonstrate that anticonvulsant, but not convulsant, succinimides block LTCC, provide additional support for the hypothesis that LTCC reduction is a mechanism of action of the anticonvulsant succinimides related to their effects in petit mal epilepsy.

## Introduction

Spike-wave discharges, the electroencephalographic hallmark of petit mal seizures, are thought to result from an underlying oscillatory interaction between reciprocally connected thalamic and cortical neuronal populations in both animal models of petit mal (Avoli *et al.*, 1983; Gloor & Fariello, 1988; Vergnes *et al.*, 1987), and in man with this form of epilepsy (Williams, 1953). Virtually all thalamic neurones are endowed with a prominent low-threshold calcium conductance that is of sufficient magnitude to generate a low-threshold calcium spike (Deschênes *et al.*, 1984; Jahnsen & Llinás, 1984a,b; Coulter *et al.*, 1989c). This conductance is important in the generation of normal thalamocortical oscillations such as sleep spindles (reviewed in Steriade & Llinás, 1988), and may also play a key role in the development of spike-wave discharges of petit mal (see Coulter *et al.*, 1989b for review). We have proposed that reductions in this calcium current in thalamic neurones might be one mechanism by which the thalamocortical oscillatory interaction underlying petit mal epilepsy could be dampened or blocked by anticonvulsant drugs (Coulter *et al.*, 1989a,b). Ethosuximide (ES; structure illustrated in Figure 1) is an agent widely used in treatment of petit mal (Browne *et al.*, 1975). Previous results from this laboratory have shown that ES, but not succinimide, the unsubstituted, clinically inactive ring base of ES (Ferrendelli & Kupferberg, 1980), specifically reduces the low-threshold calcium current (LTCC) underlying the low-threshold calcium spike in thalamic neurones (Coulter *et al.*, 1989a,b). A structurally similar petit mal anticonvulsant, dimethadione (DMD), also reduced LTCC in thalamic neurones, when applied in clinically relevant concentrations (Coulter *et al.*, 1989a,b; Figure 1).

The reduction of LTCC in thalamic neurones may thus be a cellular mechanism by which ES and DMD control petit mal seizures. In order to test this hypothesis further, we have examined the actions of ES, two other succinimides ( $\alpha$ -methyl- $\alpha$ -phenyl succinimide, an anticonvulsant, MPS; Figure 1), tetramethylsuccinimide (TMS, a convulsant; Figure 1) and the

convulsant pentylenetetrazol (PTZ) on calcium currents and, in an accompanying paper (Coulter *et al.*, 1990), on  $\gamma$ -aminobutyric acid (GABA)ergic responsiveness of thalamic neurones. The convulsant action of the  $\alpha$ -,  $\beta$ -substituted succinimide, TMS (Klunk *et al.*, 1982c), may be due to its reduction of GABAergic responsiveness in cortical neurones (Barnes & Dichter, 1984). A similar mechanism may be responsible for the convulsant action of PTZ (Nicoll & Padjen, 1976; Macdonald & Barker, 1977). PTZ-induced convulsions are a commonly utilized animal model of epilepsy against which drugs are tested for potential petit mal anticonvulsant activity. Many drugs effective in the control of petit mal are also effective against PTZ seizures, including ES, MPS, and DMD. However, some drugs which are ineffective in petit mal (e.g. phenobarbitone and primidone) are of equal or greater potency in blocking PTZ seizures than petit mal

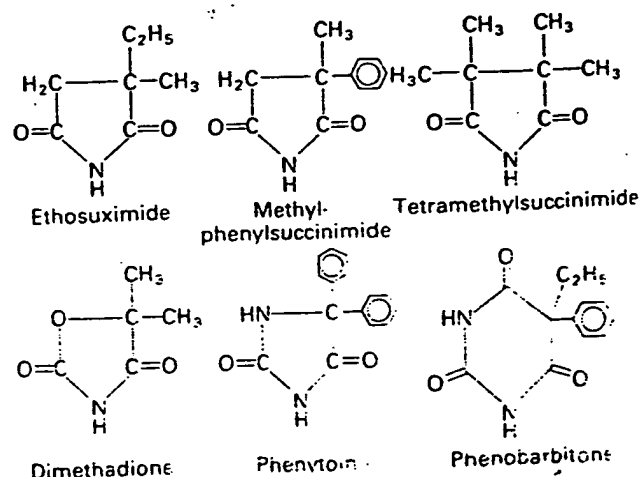


Figure 1 Structures of anticonvulsant and convulsant drugs discussed in the text. Top row: three succinimide drugs, two anticonvulsants (ethosuximide and methyl-phenyl succinimide) and one convulsant (tetramethylsuccinimide). Bottom row: three anticonvulsant drugs (dimethadione, phenytoin, and phenobarbitone).

anticonvulsants (Löscher & Schmidt, 1988). We were therefore interested in the effects and interactions of petit mal anticonvulsants on calcium currents and on GABA responses of thalamic neurones (Coulter *et al.*, 1990).

We hypothesized that TMS and PTZ should exhibit low potency in reducing LTCC (a proposed petit mal anticonvulsant action) and higher potency in blocking GABAergic responsiveness (a proposed convulsant action), while MPS should reduce LTCC in clinically relevant concentrations (as does ES), and exhibit lower potency as a blocker of GABAergic responsiveness (as should ES). These spectra of action are supported by the results presented here, and in the accompanying paper (Coulter *et al.*, 1990). In addition, we found that the block of LTCC produced by MPS had many similarities to that produced by ES. The implication of these findings with respect to the potential link between LTCC reduction and petit mal anticonvulsant action is discussed, as is the structure-activity relationship of the LTCC-blocking receptor.

## Methods

### Dissociation

All experiments were performed on thalamic neurones isolated from the ventrobasal nucleus of young rats (age 1–15 days) with methods described by Kay & Wong (1986), modified as described in Coulter *et al.* (1989c). Animals were anaesthetized with pentobarbitone, decapitated, and a block of brain containing the ventrobasal nucleus of the thalamus was removed. This tissue block was glued to the stage of a vibratome (Lancer, St. Louis, MO, U.S.A.) and sliced into 500  $\mu$ m sections. Slices were then placed into oxygenated, piperazine-N, N'-bis(2-ethanesulphonic acid) (PIPES)-buffered solution containing trypsin (0.8 mg ml<sup>-1</sup>, Sigma, St. Louis, MO, U.S.A.) for 45–100 min. The PIPES solution contained (in mM): NaCl 120, KCl 5, CaCl<sub>2</sub> 1, MgCl<sub>2</sub> 1, D-glucose 25, PIPES 20 (pH 7.0). Slices were then removed from the enzyme solution, rinsed, cut into chunks, and triturated with fire-polished Pasteur pipettes. Cells were plated onto culture dishes (Lux, E & K Scientific, Saratoga, CA, U.S.A.), and stored in an oxygenated chamber for 0.5–12 h before use.

### Voltage-clamp recording

Whole-cell voltage-clamp recordings of calcium currents were made with the method described by Hamill *et al.* (1981). The intracellular (pipette) solution contained (in mM): Trizma phosphate (dibasic) 110, Trizma base 28, ethylene glycol bis-( $\beta$ -aminoethylether)-N,N,N',N'-tetraacetic acid 11, MgCl<sub>2</sub> 2, CaCl<sub>2</sub> 0.5 and Na<sub>2</sub>-ATP 4; pH 7.35. The pipette solution also contained an intracellular ATP reconstitution system consisting of creatine phosphokinase 50 U ml<sup>-1</sup>, and phosphocreatine 22 mM (Forscher & Oxford, 1985; Mody *et al.*, 1988). This maintenance solution was used to fill the shank of the electrode, but was omitted from the solution which was used to back-fill the tip of the electrode, because it was found that gigaohm seals were difficult to obtain with protein in the tip solution. The external solution contained (in mM): NaCl 155, KCl 3, MgCl<sub>2</sub> 1, CaCl<sub>2</sub> 3, HEPES-Na<sup>+</sup> 10 and tetrodotoxin 0.0005; pH 7.4. All recordings were obtained at room temperature (20–22°C). Patch electrodes were pulled on a List LM-3P-A puller by a two-stage pull, and had resistances of 6–8 M $\Omega$ . Currents were monitored with an Axopatch 1A amplifier (Axon Instruments, Burlingame, CA, U.S.A.) and filtered at 5 kHz with an 8-pole Bessel filter before digitization. All data were stored and analysed by a DEC PDP 11/73 computer, with a Cheshire data interface (Indec, Sunnyvale, CA, U.S.A.). Leak and capacitive currents were subtracted from active currents in all figures (Coulter *et al.*, 1989c).

### Drug concentrations and method of application

MPS is an N-demethylated active metabolite of methsuximide (Celontin, Parke Davis) (Strong *et al.*, 1974; Porter *et al.*, 1979) that is effective in the control of petit mal epilepsy. It is more toxic than ES (Chen *et al.*, 1963), but has a broader spectrum of action, being also effective against complex partial seizures (Wilder & Buchanan, 1981; Browne *et al.*, 1983). Clinically relevant free serum concentrations of MPS are in the range of 50–250  $\mu$ M (Strong *et al.*, 1974; Porter *et al.*, 1979), while those for ES have been found to be 250–750  $\mu$ M (Browne *et al.*, 1975). We applied MPS (Sigma, St. Louis, MO, U.S.A.) and ES (a gift of Parke Davis, Ann Arbor, MI, U.S.A.) in a concentration range of 10  $\mu$ M to 10 mM to explore fully the dose-dependence of the LTCC reduction by these agents. TMS (ICN Biomedicals, Inc. Cleveland, OH, U.S.A.) free serum concentrations in man are obviously unavailable. However, TMS reduces GABAergic responses by 60–80% at 0.5–1 mM in cultured cortical neurones (Barnes & Dichter, 1984), and therefore we applied the drug concentrations of 10  $\mu$ M to 10 mM. ES and TMS were readily soluble in the perfusion medium. MPS often required sonication to speed solubilization. All drugs were bath applied.

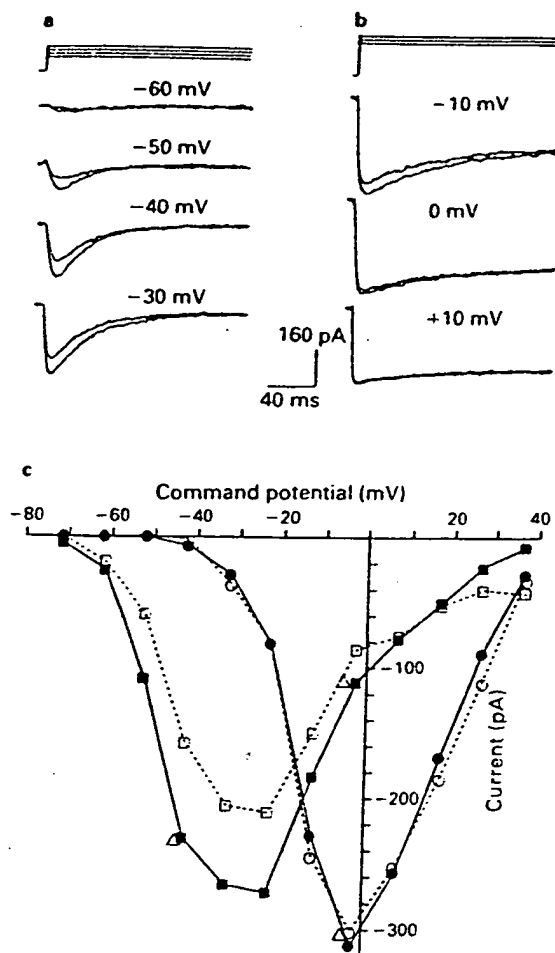
## Results

### Calcium currents

Under voltage-clamp conditions, with internal and external ionic composition designed to isolate calcium currents (see Methods), depolarizing voltage commands elicit at least two distinct calcium currents in thalamic neurones, which differ both in their kinetics, and sensitivity to pharmacological blockers (Coulter *et al.*, 1989c). Small depolarizing commands from a holding potential of –100 mV evoked a transient, fully inactivating current that could be seen in isolation (e.g. Figure 2a). This current had a threshold of approximately –65 mV. Larger amplitude depolarizing commands elicited a high-threshold, sustained component of calcium current (HTCC), with a threshold of approximately –30 mV (Figure 2b). The HTCC peaked in amplitude during step commands to 0 mV (e.g. Figure 2b and c), and varied little in magnitude when evoked from holding potentials of –100 mV or –30 mV (see Coulter *et al.*, 1989c). By contrast, the low threshold, transient calcium current (LTCC) showed complete steady-state inactivation at potentials of –65 mV or more depolarized, and was half-inactivated at –83 mV, on average (Coulter *et al.*, 1989c). As in other neurones (Fox *et al.*, 1987; Narahashi *et al.*, 1987), the LTCC and HTCC were differentially blocked by divalent cations, with the LTCC being more sensitive to Ni<sup>2+</sup>, and the HTCC to Cd<sup>2+</sup> (Coulter *et al.*, 1989c). Therefore, the LTCC appears analogous to the 'T' current (Fox *et al.*, 1987), 'type I' current (Narahashi *et al.*, 1987) or the 'l.v.a.' current (Carbone & Lux, 1984) described by others while the HTCC has properties similar to the 'L' current (Fox *et al.*, 1987), 'type II' current (Narahashi *et al.*, 1987), or the 'h.v.a.' current (Carbone & Lux, 1984).

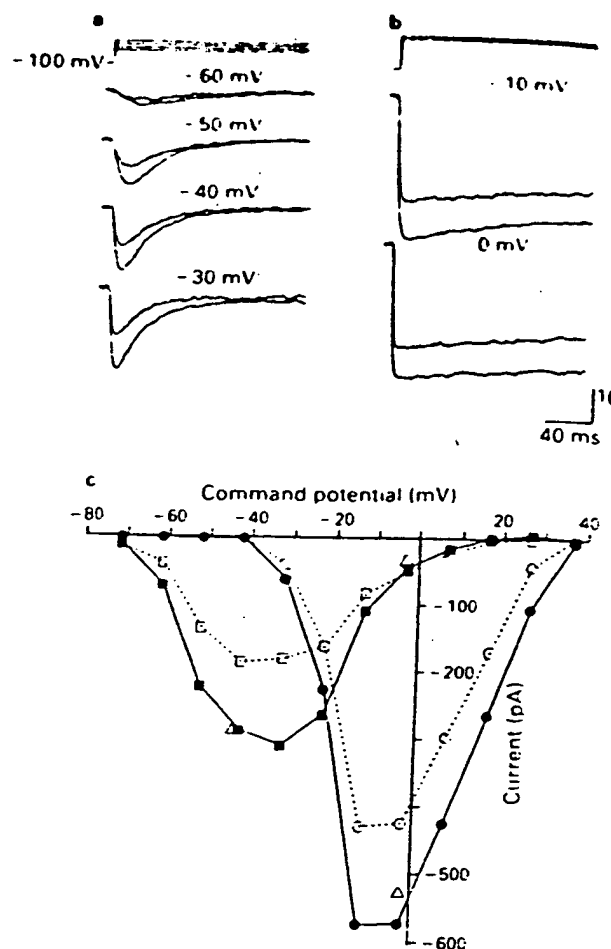
### Drug effects on calcium currents

The actions of drugs on calcium currents were examined in 77 neurones. MPS (20  $\mu$ M–20 mM) reduced the LTCC in all neurones to which it was applied ( $n = 55$ ). In higher concentrations ( $\geq 500 \mu$ M), it reduced the high threshold calcium current (HTCC) as well. Figure 2 illustrates the selective reduction of LTCC (a) without effects of the sustained HTCC (b) in response to bath application of 200  $\mu$ M MPS. In Figure 2c, amplitudes of the transient and sustained calcium currents are plotted vs command potential eliciting the current in control, MPS 200  $\mu$ M, and wash conditions, to show the reversibility of the drug action, and its selectivity for the LTCC at this con-



**Figure 2** Effects of methylphenylsuccinimide (MPS, 200  $\mu$ M) on calcium currents in a thalamic neurone. (a,b) Two superimposed traces at various depolarizing command potentials, from a holding potential of  $-100$  mV, show the effects of MPS on the transient low-threshold calcium current (LTCC) and the sustained high-threshold calcium current (HTCC). The larger peak current in each case is the control. This concentration of MPS specifically reduces the LTCC. (c) Plot of voltage command potential vs transient and sustained calcium current amplitude, under control, MPS-exposed (200  $\mu$ M), and wash conditions for the cell of (a). Transient current was defined as the portion of current that decayed during the 200 ms duration of the step command, and the sustained current was defined as the portion of current which remained at the end of the 200 ms step command. At this concentration, MPS specifically reduced the transient, low-threshold calcium current over the full activation range of this current, and this effect was fully reversible. (■) Control transient current, (□) transient current in presence of 200  $\mu$ M MPS; (●) control sustained current, (○) sustained current in the presence of 200  $\mu$ M MPS;  $\Delta$  recovery.

centration. Figure 3 illustrates the effects of bath application of 800  $\mu$ M MPS on calcium currents in another neurone. At this concentration, MPS reduced both the LTCC (Figure 3a) and the HTCC (Figure 3b). This effect is also shown graphically in Figure 3c. The traces of Figures 2 and 3 show that this reduction of calcium current occurred without effects on the time-course of the current. A concentration-response relationship for the effects of MPS on LTCC was plotted (semilogarithmically) in Figure 4. The resulting data plot can be fitted with a function that assumes a bimolecular interaction between drug and receptor, a maximal effect of 100%, and an  $IC_{50}$  of 1100  $\mu$ M. The corresponding Hill plot of Figure 4b is best fitted by a linear regression with a slope of  $0.79 \pm 0.05$  ( $r = 0.98$ ), also supporting a bimolecular interaction between MPS and its receptor. Co-operative binding of more than one molecule of MPS to the LTCC-reducing receptor would result in a Hill plot with a slope of  $> 1$ .



**Figure 3** Effects of methylphenylsuccinimide (MPS, 800  $\mu$ M) calcium currents in a thalamic neurone. (a,b) Sweeps as described in the legend of Figure 2. The larger peak current in each case is control. Depolarizing command level eliciting the current accompanies each trace. MPS, at this concentration, reduced both the transient low-threshold calcium current (LTCC) and the sustained high-threshold calcium current (HTCC). (c) Plot of voltage command amplitude vs transient (■, □) and sustained (●, ○) calcium current amplitude, under control (■, ●), MPS (800  $\mu$ M)-exposed (□, ○), wash ( $\Delta$ ) conditions for the cell of (a). Transient and sustained currents are defined as in the legend of Figure 2. MPS, at this high concentration, reduced both the transient, low-threshold calcium current and the high threshold, sustained calcium current over the activation range of these currents in a completely reversible manner.

The reductions of LTCC produced by MPS were strongly influenced by the command potential used to elicit current. This voltage-dependence is illustrated in Figure 5a. Figure 5a shows the reduction of LTCC elicited in response to varying voltage commands from  $-70$  to  $-35$  mV (holding potential =  $-102$  mV) in a cell exposed to 800  $\mu$ M MPS. LTCC was elicited in isolation over this range of voltage commands (Figures 2 and 3; see also Coulter *et al.*, 1989c). Fractional MPS reduction of the LTCC was maximal for currents evoked by less depolarized command potentials. For example, MPS (800  $\mu$ M) blocked 88% of the LTCC evoked by a step to  $-65$  mV, while only 42% reduction occurred when the LTCC was elicited by a command step to  $-35$  mV. Figure 5b shows a plot of the percentage reduction of LTCC vs. command potential used to elicit the LTCC for the cell of Figure 5a. With lower, clinically relevant concentrations of MPS (2–250  $\mu$ M) the voltage-dependence of reduction was also pronounced. For example, in 5 cells, MPS (250  $\mu$ M) was significantly more effective in blocking the LTCC-elicited by a step to  $-60$  mV ( $22.2 \pm 2.8\%$  reduction, mean  $\pm$  s.d.) than LTCC elicited by a step to  $-30$  mV ( $7.4 \pm 3.9\%$  reduction,  $t$  test,  $P < 0.0001$ ,  $t = 6.90$ , degrees of freedom = 8). Figure

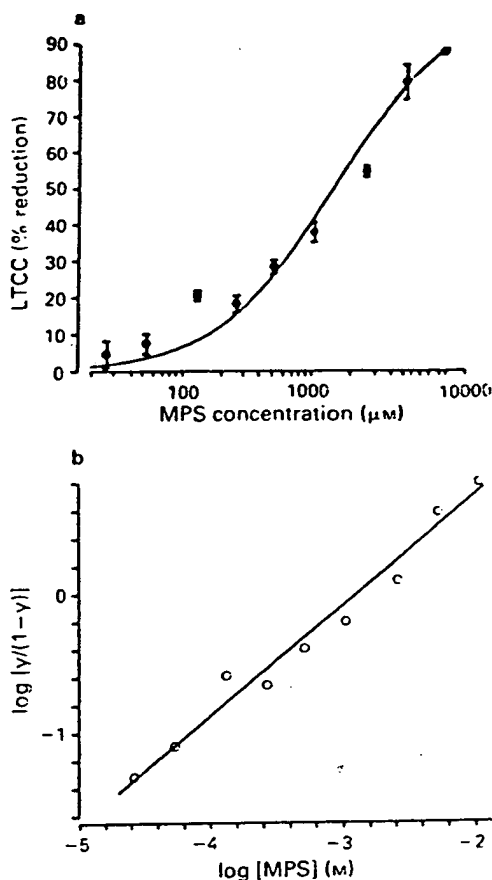


Figure 4 (a) Plot of methylphenylsuccinimide (MPS) bath concentration (logarithmic scale) vs % reduction of low-threshold calcium current (LTCC) ( $V_{\text{hold}} = -100\text{ mV}$ , command potential =  $-40\text{ mV}$ ) for 31 thalamic neurones (symbols show mean and vertical lines s.e. mean for 3–10 cells per point). The curve, which was fitted to the data by eye, was constructed from the equation: effect = maximal effect  $\times$  MPS concentration / (MPS concentration +  $\text{IC}_{50}$ ). An  $\text{IC}_{50}$  estimate of  $1100\text{ }\mu\text{M}$  was used, and a maximal effect estimate of 100%. (b) Hill plot of log MPS concentration vs log [effect/(1 - effect)]. The line was fitted to the data by linear regression, and has a slope of 0.79 ( $r = 0.98$ ). Both the equation of (a), and the slope of the line in (b) support a bimolecular interaction between drug and receptor.

and d illustrate the voltage-dependent reduction of LTCC in response to  $200\text{ }\mu\text{M}$  MPS.

One mechanism for reductions in LTCC by MPS might be an effect on the voltage-dependent steady-state inactivation of this current. This possibility was examined by evoking the LTCC by a depolarizing step to  $-42\text{ mV}$  from different holding potentials as shown in Figure 6a. MPS exposure did not alter the voltage-dependence of steady-state inactivation, although it reduced the peak amplitude of the LTCC. Figure 6b illustrates a plot of the average fraction of LTCC available to be activated (relative to the maximal LTCC under control and drug-exposed conditions) vs the holding potential from which the LTCC was evoked, for 4 cells to which MPS ( $800\text{ }\mu\text{M}$ ) was bath applied, and shows that drug application had no effect on steady-state inactivation of the LTCC relative to control. In addition, lower, clinically relevant concentrations of MPS were also without effect (not shown).

Many aspects of MPS reduction of LTCC are similar to those previously demonstrated for ES (Coulter *et al.*, 1989b). We therefore compared the effects of these two agents (in equivalent concentrations) on calcium currents in 6 cells. In this group of neurones,  $500\text{ }\mu\text{M}$  ES or MPS reduced LTCC by  $23.4 \pm 2.7\%$  and  $27.6 \pm 3.6\%$ , respectively. By contrast in 14 cells, the convulsant succinimide TMS had little effect on LTCC at concentrations of  $1\text{ mM}$  or less (0% reduction at  $100\text{ }\mu\text{M}$ ,  $4.4 \pm 2.0\%$  reduction at  $1\text{ mM}$ , in 4 cells to which concentrations of  $100\text{ }\mu\text{M}$ ,  $1\text{ mM}$ , and  $10\text{ mM}$  TMS were applied).

Figure 7 illustrates the effect of  $1\text{ mM}$  MPS and TMS, on LTCC in the same neurone. MPS clearly reduced the LTCC in a reversible manner, while TMS had little or no effect on the LTCC at  $1\text{ mM}$ . In addition, Figure 7 shows that TMS, when applied together with MPS in equivalent concentrations, had no influence on MPS effects. Reductions in LTCC elicited by  $1\text{ mM}$  MPS were similar to those produced by perfusion with  $1\text{ mM}$  MPS and  $1\text{ mM}$  TMS. This finding suggests that TMS does not antagonize MPS LTCC-reducing effects by binding to the LTCC-reducing receptor without activity. At very high concentrations ( $10\text{ mM}$ ), TMS did reduce LTCC (by  $30.3 \pm 4.0\%$ ,  $n = 4$ ). The characteristics of this LTCC block by high concentrations of TMS appeared similar to those elicited by ES and MPS, including similar voltage-dependence (not shown). PTZ had no effect on LTCC in concentrations from  $100\text{ }\mu\text{M}$  to  $10\text{ mM}$  ( $n = 8$ , not shown).

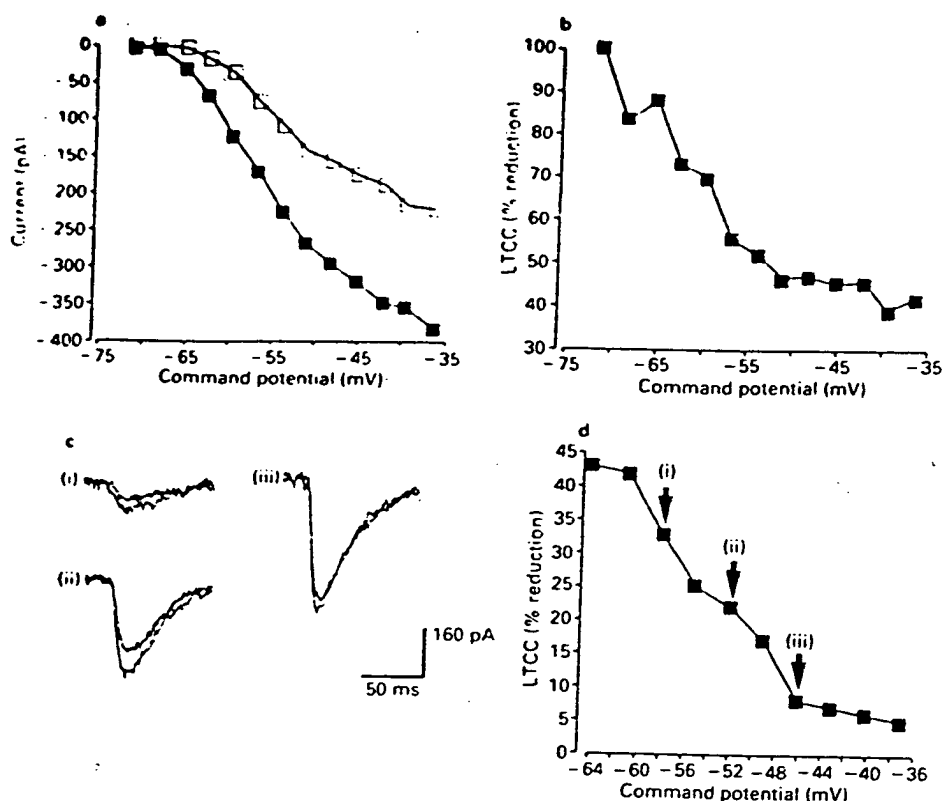
## Discussion

We previously demonstrated that both ES and DMD, structurally similar petit mal anticonvulsants (Figure 1), reduce LTCC in thalamic neurones when applied in clinically relevant concentrations (Coulter *et al.*, 1989a,b). The clinically inactive ring base of ES, succinimide, had no effect on the LTCC at equivalent concentrations (Coulter *et al.*, 1989a). The results of the experiments described here show that another anticonvulsant succinimide, MPS, also reduces LTCC in these cells, while a convulsant succinimide, TMS, only reduces LTCC when applied in very high concentrations. PTZ, a convulsant commonly utilized to screen drugs for potential petit mal anticonvulsant activity, did not affect calcium currents in concentrations up to  $10\text{ mM}$ .

Thalamic neurones are thought to participate in the oscillatory thalamocortical discharge underlying the 3 Hz spike-wave rhythms of petit mal. The low-threshold calcium spike, which is generated by the LTCC (Coulter *et al.*, 1989c) and is ubiquitous in thalamic neurones, has been implicated as important in the generation and maintenance of thalamocortical oscillatory interactions (reviewed in Steriade & Llinás, 1988). The reduction in LTCC produced by ES, MPS, and DMD therefore appears compatible with the anticonvulsant activity of these compounds (i.e. they may depress the pathological thalamocortical rhythm underlying the spike-wave discharges through their effects on LTCC). The lack of effect of the inactive and convulsant succinimides, succinimide and TMS, on LTCC also appears consistent with this conclusion.

MPS-induced reduction of LTCC is similar in many respects to ES reduction of the same conductance. Like ES, MPS reduces LTCC without affecting the time course of the current (Coulter *et al.*, 1989b), suggesting that the LTCC reduction is accomplished without either slowing activation or speeding inactivation (Figure 2a,b). The actions of MPS and ES occur without effects on the voltage-dependence of steady-state inactivation (Figure 6; Coulter *et al.*, 1989b), and both agents reduce LTCC in a similar voltage-dependent manner, with maximal effects at the voltage threshold for eliciting the current (Figure 5; Coulter *et al.*, 1989b). Such voltage-dependence would increase the effectiveness of these drugs *in vivo*, by reducing the LTCC maximally when it is elicited by small depolarizations, as would occur at the onset of spindle waves (Steriade & Llinás, 1988) and probably during spike wave discharges (SWD). In a positive feedback circuit such as the thalamocortical loop underlying the spike-and-wave discharge of petit mal, this kind of action would be very effective either at blocking the onset of the discharge, or severely curtailing the full expression of the oscillations responsible for the epileptic attack.

There were also some interesting differences between the actions of ES and MPS. ES reduced LTCC with greater potency, but lower efficacy than MPS (the  $\text{IC}_{50}$  for ES was  $200\text{ }\mu\text{M}$  vs  $1100\text{ }\mu\text{M}$  for MPS and the maximal effects were 40% and 100% reductions for ES and MPS, respectively (Coulter *et al.*, 1989b; Figure 2c)). MPS was also less specific than ES



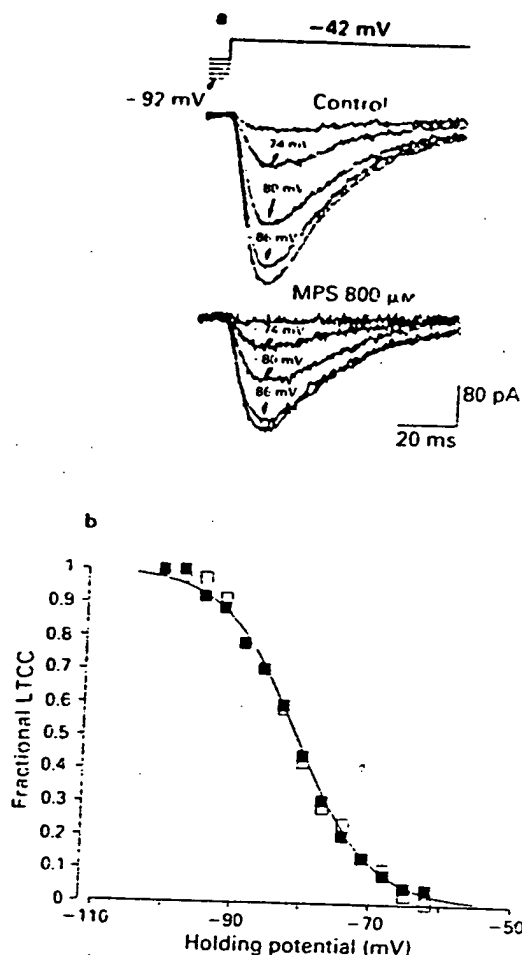
**Figure 5** Voltage-dependence of methylphenylsuccinimide (MPS)-induced reduction of the low-threshold calcium current (LTCC). (a) Plot of amplitude of calcium current vs command potential in the potential range where the LTCC is elicited in isolation, under control (■) and MPS (800  $\mu$ M)-exposed (□) conditions. There is a pronounced voltage-dependence to MPS reduction of the LTCC; proportionally larger reductions of LTCC occurred when the current was elicited near threshold than at more depolarized command potentials. (b) Plot of percentage reduction of LTCC by MPS (800  $\mu$ M) vs command potential for the same cell as in (a). (c) Effects of MPS (200  $\mu$ M) on LTCC evoked by 3 different command potentials (i) = -58 mV, (ii) = -52 mV, (iii) = -46 mV in a different cell from that in (a) and (b). Control and MPS-exposed traces are superimposed. MPS produced a proportionately greater reduction of LTCC at less depolarized step commands. (d) Plot of amplitude of calcium current vs command potential in the potential range where the LTCC was elicited in isolation, under control and MPS (200  $\mu$ M)-exposed conditions for the same cell as in (c). At this concentration, the voltage-dependence of LTCC reduction was qualitatively similar but quantitatively smaller than that obtained when 800  $\mu$ M MPS was applied (a and b). The potentials corresponding to the traces in (c) are indicated by arrows.

in reducing calcium currents. ES selectively reduced LTCC, rarely affecting the HTCC at any concentration (Coulter *et al.*, 1989a,b), while MPS had a selective action on LTCC at clinically relevant concentrations (200  $\mu$ M or less, Figure 3a,b) but reduced the HTCC as well at higher concentrations (Figure 3c). This effect of higher concentrations of MPS was similar to that of DMD (Coulter *et al.*, 1989a,b). This decreased specificity of MPS and DMD for the LTCC might contribute to their increased clinical toxicity relative to ES. The HTCC-reducing effects of MPS and DMD are probably not related to their petit mal anticonvulsant effectiveness, since clinically relevant concentrations of MPS block only LTCC. Also, ES, which is clinically selective for petit mal, does not reduce HTCC.

Evidence from these experiments and other studies provides insight into the structural aspects of the receptor that mediates LTCC reduction. ES and MPS (both  $\alpha$ -substituted succinimides, Figure 1) reduce LTCC at concentrations less than 1 mM, while succinimide (the unsubstituted ring base of ES and MPS) does not (Coulter *et al.*, 1989a), suggesting that some alkyl substitutions are necessary for activity at this receptor. TMS, an  $\alpha$ - and  $\beta$ -substituted succinimide, does not reduce LTCC to any significant extent unless applied at high concentrations ( $\geq$  1 mM), and does not occlude MPS responses when applied at 1 mM. This suggests that TMS binds with very low affinity (relative to ES and MPS) to the LTCC-reducing receptor. Furthermore, since TMS does not occlude MPS reduction of LTCC when applied concurrently (Figure 7), it is unlikely that TMS even binds to any significant degree to the receptor at 1 mM concentration. Therefore,  $\alpha$ -substitution appears necessary for activity at the LTCC-

reducing receptor, while  $\beta$ -substitution reduces effectiveness. DMD and phenytoin also reduce the LTCC (Twombly *et al.* 1988; Coulter *et al.*, 1989a,b). These molecules are similar to ES and MPS in that they are substituted 5-membered heterocyclic rings with two carbonyl carbons bordering a nitrogen but they differ in their ring closure (-N- for phenytoin, -O- for DMD, vs -CH<sub>2</sub>- for the succinimides, Figure 1). This suggests that there is some nonselectivity on the part of the receptor terms of the ring closure, and ring shape of agonists. One prediction from this is that certain barbiturates, including phenobarbital, which are structurally similar to the act succinimides (particularly to MPS, Figure 1) might be active at the LTCC-reducing site. This prediction is currently being tested.

Many characteristics of this LTCC-reducing receptor are similar to those of a receptor responsible for anticonvulsant activity described by Ferrendelli and colleagues (Klunk *et al.* 1982c). In their studies of alkyl-substituted butyrolactones and succinimides, these investigators showed that  $\alpha$ -substitution alone resulted in significant anticonvulsant activity (Klunk *et al.*, 1982b), while  $\alpha$ - and  $\beta$ - or  $\gamma$ - (for the butyrolactones) substitution resulted in convulsant activity (Klunk *et al.*, 1982). The order of potency of the various succinimides in reducing LTCC is ES > MPS > TMS, while the order of potency of these same compounds in reducing GABAergic responsiveness is TMS > ES > MPS (Coulter *et al.*, 1990). The receptor mediating the LTCC-reducing action (as discussed above) appears to be distinct from the picrotoxin receptor, hypothesized by Klunk *et al.* (1982c) to be responsible for the convulsant actions of TMS. PTZ, which shares the convulsant actions of TMS, perhaps inducing convulsions by a similar



**Figure 6** Steady-state inactivation of control and methylphenylsuccinimide (MPS)-reduced low-threshold calcium current (LTCC). (a) Superimposed sweeps of control (top) and MPS (800  $\mu$ M)-exposed (bottom) LTCC, evoked by depolarizing commands to -42 mV from varying holding potentials (upper traces). Sweeps corresponding to holding potentials of -86, -80, and -74 mV, the steepest portion of the steady-state inactivation curve, are labelled under both conditions. MPS exposure did not alter the voltage-dependence of steady-state inactivation (although it reduced the peak amplitude of the LTCC), and the time-course of the LTCC was not affected by its amplitude. (b) Plot of holding potential vs mean fraction of LTCC available (relative to LTCC evoked from -112 mV) from 4 cells recorded under control (■) and MPS (800  $\mu$ M)-exposed (□) conditions (includes the cell of (a)). MPS reduced LTCC without affecting the voltage-dependence of steady-state inactivation.

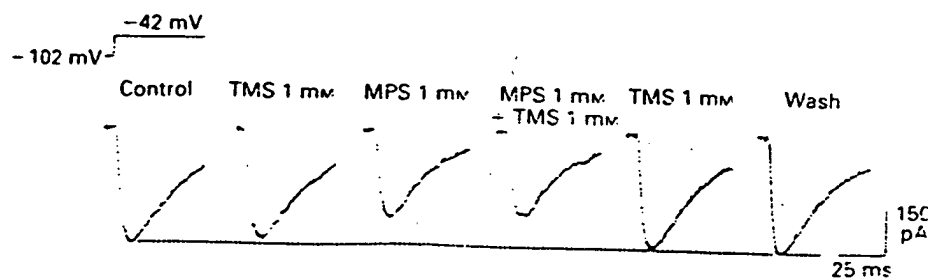
mechanism, GABAergic blockade (Nicoll & Padjen, 1976; Macdonald & Barker, 1977), did not affect LTCC in concentrations up to 10 mM, and so its convulsant actions cannot be attributed even partially to actions on thalamic calcium cur-

rents. These findings have important implications related to the use of PTZ-induced seizures as a screen for testing potential petit mal anticonvulsants, and are further discussed in the succeeding paper (Coulter *et al.*, 1990).

The LTCC reducing effects of MPS do not explain its anticonvulsant spectrum of action, since this drug is effective against both petit mal and other seizure types, whereas ES, with essentially the same action on LTCC, is selectively effective in petit mal absence. MPS is structurally intermediate between ES and phenytoin in that it has both alkyl and phenyl substitutions at what corresponds to the  $\alpha$ -position on a heterocyclic-5-membered ring (Figure 1). Phenytoin is known to reduce sustained repetitive firing (reviewed in Macdonald & McLean, 1986), and to shift steady-state inactivation of the sodium current (Willow *et al.*, 1985), actions which may underlie its anticonvulsant effects against partial complex and generalized motor seizures (Macdonald & McClean, 1986). These actions of phenytoin, together with the structural similarities between MPS and phenytoin, lead to the hypothesis that the broader spectrum of action of MPS versus ES might be due to effects of the former drug on sodium currents.

One interesting consequence of the hypothesis that thalamic low-threshold calcium spikes are involved in generation of spike-wave discharge is that drugs which specifically block the LTCC and cross the blood/brain barrier might be expected to exhibit petit mal anticonvulsant activity. Various alcohols, including menthol (Swandulla *et al.*, 1987), and octanol (Llinás & Yarom, 1986) have been shown to block LTCC in different neuronal preparations. However, this block is not specific, in that the HTCC is also reduced (Swandulla *et al.*, 1987; Twombly & Narahashi, 1989). It also remains to be determined whether these alcohols have added effects on other ion channels, and/or on membrane fluidity. Another interesting family of drugs which might have anticonvulsant activity are amiloride and related compounds. These agents specifically block LTCC in mouse neuroblastoma and chick dorsal root ganglion neurones (Tang *et al.*, 1988). However, other actions include inhibition of the  $\text{Na}^+/\text{H}^+$  and  $\text{Na}^+/\text{Ca}^{2+}$  exchange systems (Kinsella & Aronson, 1981; Schellenberg *et al.*, 1983), and blockade of the tetrodotoxin-insensitive  $\text{Na}^+$  channel in epithelial cells (Palmer, 1984). Also, the positive charge on amiloride at physiological pH should prevent the drug from crossing the blood/brain barrier. It may be possible to develop analogues of amiloride, or entirely new compounds, which will cross the blood/brain barrier, block LTCC, and prove effective as anticonvulsants.

The finding that petit mal anticonvulsant succinimides ES and MPS, but not convulsant (TMS) and inactive succinimides (succinimide), reduce the LTCC in thalamic neurones in clinically relevant concentrations lends strong support to the idea that this cellular action is related to the clinical effectiveness of these agents as anticonvulsants. This idea is further supported by the finding that the convulsant PTZ was without effect on calcium currents in concentrations up to 10 mM.



**Figure 7** Effects of tetramethylsuccinimide (TMS), methylphenylsuccinimide (MPS) and MPS plus TMS on the low-threshold calcium current (LTCC) in one neurone. Sweeps illustrating the amplitude of LTCC in control, 1 mM TMS, 1 mM MPS, 1 mM TMS plus 1 mM MPS and 1 mM TMS again, and wash conditions. TMS had little or no effect on LTCC at 1 mM, and did not occlude MPS reductions of LTCC when applied concurrently. All currents were elicited by a step command to -42 mV from a holding potential of -102 mV. The dotted line is included to aid comparison between sweeps.

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